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Bioconjugates of Chelators with Peptides and Proteins in Nuclear Medicine: Historical Importance, Current Innovations and Future Challenges

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Abstract

Molecular radiopharmaceuticals based on bioconjugates of chelators with peptides and proteins have had significant clinical impact in diagnosis and treatment of several types of cancers. In the 1990s, indium-111 and yttrium-90 labelled chelator-peptide/protein conjugates established the clinical utility of these radiopharmaceuticals for receptor-targeted γ -scintigraphy imaging and systemic radiotherapy. Second generation bioconjugates based on peptides targeting the somatostatin II receptor and the prostate specific membrane antigen are now widely used for management of neuroendocrine and prostate cancer respectively. These bioconjugates are typically radiolabelled with gallium-68 for imaging of target receptor expression with Positron Emission Tomography, and the β^- -emitter, lutetium-177 for targeted radiotherapy. Innovations in radioisotope technology and biomolecular therapies are likely to drive the future clinical development of radiopharmaceuticals based on radiometals. New chelator-peptide and chelator-protein bioconjugates will underpin nuclear medicine advances in molecular imaging and radiotherapy.

Introduction

Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT), allow quantitative whole-body molecular imaging of cancer. One class of PET and SPECT/ γ -scintigraphy molecular radiopharmaceuticals incorporates a radioactive metal bound via a chelator attached to a peptide or protein, which targets cell-surface receptors of diseased cells. Systemic Peptide Receptor Radionuclide Therapy (PRRT) of cancer incorporates β^- or α -emitting radiometals into the same molecular architectures to deliver targeted therapeutic radiation.

Early clinical innovations based on radiolabelled indium-111 (¹¹¹In, half-life = 67 h, γ 100%) for SPECT/ γ -scintigraphy and β^- -emitting yttrium-90 (⁹⁰Y, half-life = 64 h, β^- 100%) for systemic radiotherapy established the utility of these types of diagnostic and therapeutic nuclear medicine. More recently, gallium-68 (⁶⁸Ga, half-life = 68 min, β^+ 90%, E_{\max} = 1880 keV) PET agents and lutetium-177 (¹⁷⁷Lu, half-life = 6.65 days, β^- 100%, γ 14%) β^- -emitting PRRTs have made a huge impact in prostate and neuroendocrine cancer treatment.

Chelators have been essential in advancing the use of radiometallated peptide and protein bioconjugates in clinical nuclear medicine. Here we highlight the *clinical* importance of bioconjugates of chelators with peptides and proteins in diagnostic PET/SPECT/ γ -scintigraphy imaging and systemic receptor-targeted radiotherapy. For more detailed reviews on chelator development or innovations in site-specific chelator conjugation chemistry, we direct readers to recent reviews.^{1–3} In this review, we adopt radiopharmaceutical nomenclature, in which square brackets denote the radioisotopic label of a compound.⁴ For example, “[¹¹¹In]In-DTPA-octreotide” indicates that the In-DTPA-octreotide bioconjugate has been enriched or labelled with radioactive indium-111, and “fac-[^{99m}Tc][Tc(CO)₃(OH₂)₃]⁺” indicates that the [Tc(CO)₃(OH₂)₃]⁺ complex has been enriched or labelled with radioactive technetium-99m.

1990s: Radiolabelled peptides and proteins are approved for clinical use

¹¹¹In-labelled bioconjugates have played a seminal role in the development of protein- and peptide-based radiopharmaceuticals. One of the first chelators used for incorporating indium-111 into

proteins and peptides was DTPA (diethylenetriaminepentaacetic acid, Chart 1), with prominent examples including:

1. the first FDA-approved (1992) radiolabelled antibody, “OncoScint”, which uses a DTPA derivative to incorporate γ -emitting indium-111 into an antibody targeting a tumor-associated glycoprotein expressed in colorectal and ovarian cancers.⁵ The 67 h half-life of indium-111 is well suited to studying the biodistribution of antibodies, which typically require several days to clear circulation and localise at target receptors.
2. a prostate-specific membrane antigen (PSMA) targeting antibody, “ProstaScint” (granted FDA approval in 1996) used for prostate cancer imaging, similarly incorporating indium-111 into an antibody via an appended DTPA chelator.⁶ Although ProstaScint has shown limited predictive value and specificity as a clinical prostate cancer diagnostic, it has been fundamental in the development of radionuclide molecular imaging and radiotherapy of its target, PSMA. Notably, DTPA is incorporated *site-specifically* into OncoScint and ProstaScint, using a GYK-DTPA peptide conjugate (Chart 1).⁷ Antibody glycol groups of terminal hexose units are oxidised, providing aldehyde motifs, which are then reacted with the primary amine of GYK-DTPA to furnish an imine conjugate.
3. the [¹¹¹In]InDTPA-octreotide peptide conjugate, “Octreoscan” (granted FDA approval in 1994, Chart 2). Octreotide and its second-generation derivative, octreotate, target the somatostatin II receptor (SSTR2).⁸ In combination with γ -scintigraphy or SPECT, Octreoscan has been used to diagnose and localise neuroendocrine tumors that express SSTR2 (Figure 1a). Although incidence of neuroendocrine cancer is relatively low compared to other cancers, SSTR2-targeted radiopharmaceuticals have significantly impacted clinical management of this disease.

Radiolabelling reactions of DTPA and its conjugates with indium-111, yttrium-90 and lutetium-177, proceed relatively rapidly at ambient temperature,⁹ however, the *in vivo* stability of the resulting complexes is modest, with some dissociation of the radiometal from chelator-biomolecule conjugates.¹⁰ As a result, DTPA has largely been superseded by chelators such as modified DTPA derivatives or DOTA that demonstrate lower release of the radiometal *in vivo*. Incorporating a methyl group into the ethylene backbone of DTPA sterically hinders the release of a radiometal from the chelator.^{11,12} The first FDA-approved (2002) radiotherapeutic immunoconjugate, Zevalin, used a DTPA derivative, “tiuxetan”, containing a methyl group and a pendant phenyl isothiocyanate (to enable bioconjugation via antibody lysine residues) to incorporate the β^- -emitting radiotherapeutic metal ion, yttrium-90, into a monoclonal ibritumomab antibody targeting CD20 receptors for radionuclide therapy of non-Hodgkin’s lymphoma.¹³

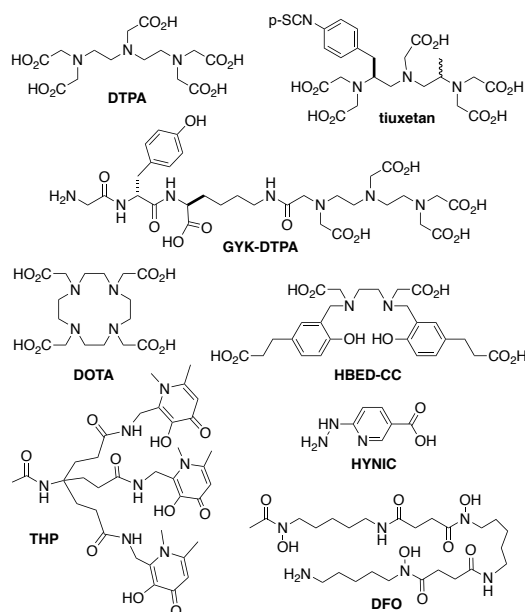


Chart 1. Chelators commonly used for labelling peptides and proteins with radiometals

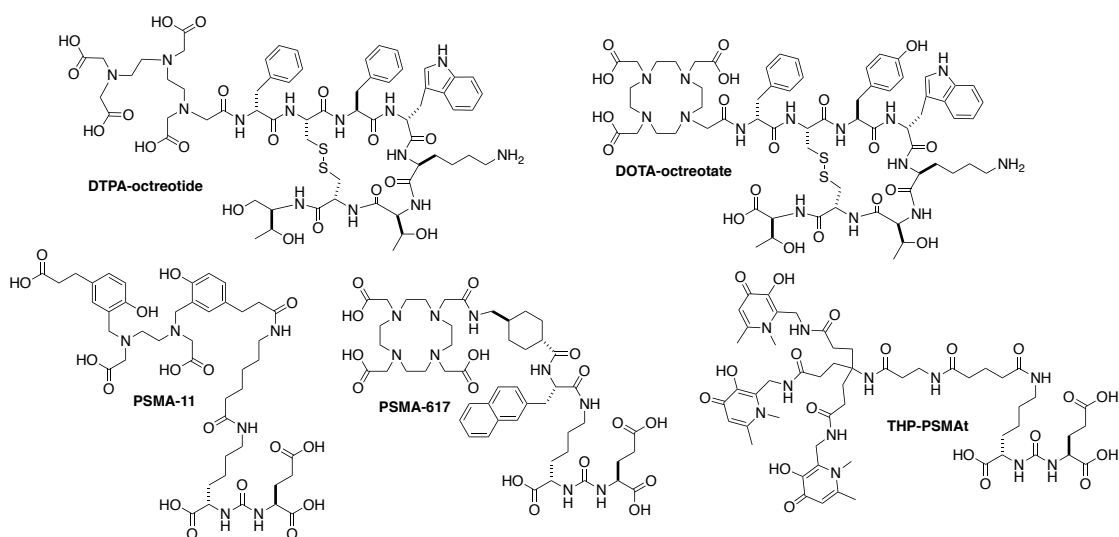


Chart 2. Chelator-peptide bioconjugates

Current state-of-the-art: chelator-peptide conjugates for receptor-targeted PET imaging and systemic radiotherapy

DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) chelator, with four tertiary amine and four carboxylate ligands, has had enormous clinical utility in nuclear medicine. DOTA's first appearance in the nuclear medicine field was in 1990 when it was found to form stable complexes with yttrium-90.¹⁴ It can coordinate a range of radiometallic isotopes, from the relatively small Ga^{3+} ion in a hexadentate N_4O_2 octahedral environment, up to lanthanide and actinide ions with large coordination spheres that adopt a range of complex geometries (sometimes including coordination of solvent molecules). The macrocyclic nature of DOTA means that it forms more thermodynamically stable and kinetically inert complexes than commonly used acyclic chelators such as DTPA. The rigid, preorganised binding site leads to a lower entropic cost of complexation, and in vivo, DOTA-based metal complexes exhibit higher kinetic stability and resist radiometal dissociation to a greater degree than, for example, DTPA.^{14,15}

As a result of this versatility, a single DOTA bioconjugate can be used for multiple applications. This has enabled the clinical development of diagnostic and therapeutic *pairs*, sometimes termed “theranostics”, in which the imaging agent is used to stage, prognose and monitor disease, and the targeted therapeutic is used to deliver a cytotoxic payload. To date, no single example better exemplifies the clinical utility of DOTA than that of its conjugates with SSTR2-targeting peptides, octreotide and its second-generation derivative, octreotate (Chart 2).

The short half-life of β^+ -emitting gallium-68 makes it suitable for PET imaging with peptides, which accumulate at target tissue and clear circulation relatively quickly (within 30 min – 1 hour). Approval of a GMP-compliant benchtop $^{68}\text{Ge}/^{68}\text{Ga}$ -generator has driven PET imaging based on ^{68}Ga -labelled peptides. Clinical studies have shown the superior diagnostic properties of [^{68}Ga]Ga-DOTA-octreotate over [^{111}In]In-DTPA-octreotide: in combination with PET, [^{68}Ga]Ga-DOTA-octreotate has better sensitivity and resolution and can detect more SSTR2-expressing tumor lesions than Octreoscan (Figure 1b),¹⁶ and the FDA approved the diagnostic use of [^{68}Ga]Ga-DOTA-octreotate in 2016.

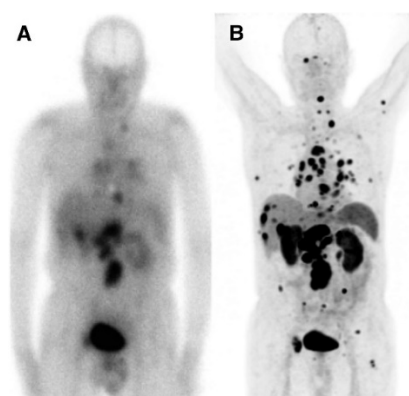


Figure 1: Head-to-head comparison of [^{111}In]In-DTPA-octreotide scintigraphy (A) and [^{68}Ga]Ga-DOTA-octreotate PET/CT (B) in a patient with metastatic low-grade neuroendocrine tumors. In liver, retroperitoneal and thoracic lymph nodes, and bones, [^{68}Ga]Ga-DOTA-octreotate PET/CT shows multiple metastases, many of which are undetectable on [^{111}In]In-DTPA-octreotide scintigraphy. This research was originally published in *Journal of Nuclear Medicine*,¹⁷ by Christophe M. Deroose et al., 2016;57:1949-1956. © SNMMI.

The advent of receptor-targeted radionuclide therapy with somatostatin analogues has changed clinical management and treatment of neuroendocrine cancer. Both DOTA-octreotide and DOTA-octreotate labelled with β^- -emitting yttrium-90 have demonstrated efficacy in systemic radiotherapy of metastatic neuroendocrine tumors,^{18,19} however, these ^{90}Y -labelled peptides have been superseded in many centres by [^{177}Lu]Lu-DOTA-octreotate. [^{177}Lu]Lu-DOTA-octreotate, known as “Lutathera”, is the first peptide-based receptor targeted radionuclide therapy to be approved by the FDA. A recent phase III randomised clinical trial of Lutathera in patients with neuroendocrine tumors has demonstrated Lutathera’s therapeutic efficacy: overall, patients receiving Lutathera experienced longer progression free survival (65.2% of patients at month 20), compared to patients in the control group (10.8% at month 20) who received therapeutic doses of non-radioactive octreotide peptide.²⁰

The success of peptide molecular imaging and systemic radiotherapy with radiolabelled somatostatin analogues, coupled with the demonstrable clinical utility of radiolabelled PSMA-targeted immunoconjugates such as ProstaScint, has led to peptide-based molecular imaging and systemic radiotherapy for prostate cancer using radiolabelled conjugates of a short, urea-containing dipeptide (referred to here as “PSMA_t”) that targets the prostate specific membrane antigen. Imaging radioisotopes, including gallium-68, and radiotherapeutic isotopes, most notably lutetium-177, have been incorporated into PSMA_t peptide conjugates via several chelators. For gallium-68 clinical PET imaging, this includes DOTA,^{21–23} a derivative of an acyclic HBED (N,N'-Bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid) chelator bearing pendant carboxylate groups^{24,25} and a THP (tris(hydroxypyridinone)) chelator (Charts 1 and 2).^{26,27} For larger radiometals such as

lutetium-177, DOTA chelator has again been employed. These PSMA-targeted DOTA conjugates have incorporated lipophilic “linker” groups between the chelating motif and the PSMA peptide,^{21–23} resulting in favourable hydrophobic interactions between the radiotracer and a known hydrophobic binding “pocket” of the PSMA receptor.

Of these chelator-PSMA conjugates, ⁶⁸Ga-labelled HBED-PSMA (known as PSMA-11) and ¹⁷⁷Lu-labelled DOTA-PSMA (known as PSMA-617²²) have been the most prevalently used radiopharmaceuticals for PET imaging and systemic radiotherapy of prostate cancer, respectively. [⁶⁸Ga]Ga-HBED-PSMA can identify very small prostate cancer lesions and has had extraordinarily high impact in clinics where it is available.²⁵ In the case of primary prostate cancer, PET imaging with [⁶⁸Ga]Ga-HBED-PSMA can stage disease and localise metastases; in the case of recurrent prostate cancer, it can localise metastases early, enabling better clinical interventions.

In ¹⁷⁷Lu-labelled DOTA-PSMA treatments, gallium-68 PET imaging (with [⁶⁸Ga]Ga-HBED-PSMA) is used (i) to determine whether or not a patient’s tumors express PSMA, and (ii) to monitor treatment response. γ -scintigraphy imaging using γ photons arising from lutetium-177 decay are also used to monitor accumulation and biodistribution of [¹⁷⁷Lu]Lu-DOTA-PSMA over cycles of therapy (Figure 2). Small clinical studies have demonstrated the benefits and efficacy of [¹⁷⁷Lu]Lu-DOTA-PSMA for treatment of metastatic prostate cancer,^{28–30} and currently, a multicentre, phase 3 randomised clinical trial (Vision trial, NCT03511664) of [¹⁷⁷Lu]Lu-DOTA-PSMA has just completed recruiting patients with metastatic prostate cancer. If this trial further evidences the success of [¹⁷⁷Lu]Lu-DOTA-PSMA observed in single centre clinical studies/trials, it will establish [¹⁷⁷Lu]Lu-DOTA-PSMA as a viable treatment for prostate cancer patients who previously would have had few therapeutic options left.

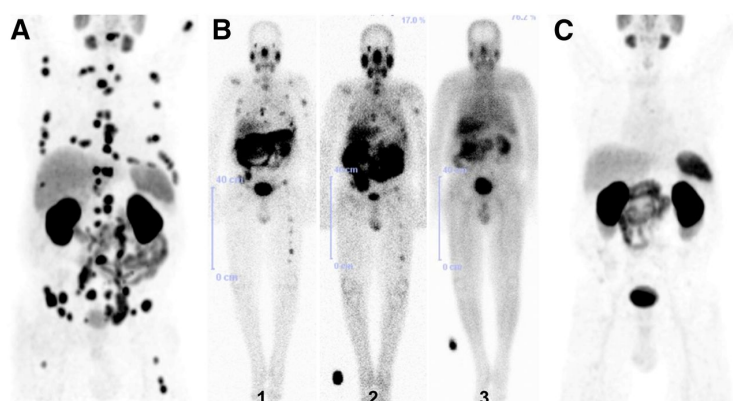


Figure 2: A 76 year old patient after external-beam radiation therapy to bone metastases and hormone therapy. (A) [⁶⁸Ga]Ga-HBED-PSMA PET/CT revealed progressive bone and lymph node metastases. (B) Lutetium-177 scintigraphy demonstrated resolution of metastases after first (1), second (2), and third (3) cycles of [¹⁷⁷Lu]Lu-DOTA-PSMA radiotherapy. (C) [⁶⁸Ga]Ga-HBED-PSMA PET/CT showed excellent molecular response with disappearance of most PSMA-avid metastases after three cycles of [¹⁷⁷Lu]Lu-DOTA-PSMA radiotherapy. This research was originally published in *Journal of Nuclear Medicine*,³⁰ by Richard P. Baum et al., 2016;57:1006-1013 © SNMMI.

Targeted radiotherapy with ²²⁵Ac-labelled bioconjugates is also an exceptionally promising field of experimental nuclear medicine. Recently, the first clinical studies using the alpha-emitter, actinium-225 (half-life = 10 days, 4 α -particles per actinium-225 atom decay), with DOTA-PSMA (PSMA-617) have been reported. In the first-in-man study of [²²⁵Ac]Ac-DOTA-PSMA, two patients with advanced metastatic prostate cancer, who had previously undergone extensive pre-therapy, experienced complete response (by PET imaging with [⁶⁸Ga]Ga-HBED-PSMA).³¹ A second study has observed similarly high efficacy of [²²⁵Ac]Ac-DOTA-PSMA in chemotherapy-naïve patients.³²

Chelator technologies to meet new clinical challenges and increase the utility, usability and availability of radiolabelled peptides and proteins

Challenges in radiolabelling

Whilst widely used, DOTA-based radiotracers have potential limitations for use as radiopharmaceutical agents due to their slow radiolabelling kinetics and high activation energies of complexation. To overcome this, heating up to 95°C is often required,³³ conditions that rule out the use of heat-sensitive bioconjugates such as antibodies. In the case of DOTA-based radiopharmaceuticals with short-lived radioisotopes – for example, ⁶⁸Ga-labelled peptides, which are typically prepared onsite at hospitals – the complexity of some of these radiolabelling procedures, which involve undesirable and prolonged heating, purification and handling,³⁴ limits the widespread availability of such radiopharmaceuticals.

Advances in chelator chemistry have focused on addressing such issues. Radiolabelled conjugates of DOTA with antibodies have been prepared by first radiolabelling a DOTA chelator derivative with a radiometallic ion, and *then* conjugating the radiolabelled DOTA derivative with an antibody.^{14,35} For example, a pre-labelled [²²⁵Ac]Ac-DOTA-tetrazine derivative has been reacted with a pancreatic cancer-targeting 5B1-antibody containing trans-cyclooctene (TCO) groups, via a bioorthogonal inverse electron demand Diels-Alder reaction.³⁵ The resulting [²²⁵Ac]Ac-DOTA-5B1 antibody shows high receptor-mediated uptake in pancreatic cancers in a mouse model. Importantly, the radiochemical yields for this process are 35 - 45%, higher than that obtained for typical actinium-225 radiolabelling reactions of DOTA-antibody conjugates (typically ~10%).

New chelators that enable fast, stable and quantitative radiometal complexation at near neutral pH, room temperature and low concentrations of chelator-bioconjugate can circumvent complicated radiolabelling protocols that involve prolonged heating and purification procedures. Many chelators have been developed that fulfil these criteria for a range of metal ions, from small, short-lived gallium-68 to large, long-lived actinium-225.^{36–39} These chelators can enable production of radiopharmaceuticals using “kits”, in which the chelator-bioconjugate, buffer components and stabilisers are incorporated into a lyophilised vial.^{26,36} The radiotracer is simply formulated by addition of a solution containing the radioactive isotope into the kit.

The hexadentate O₆ tris(3-hydroxypyridin-4-one) (THP, Chart 1) chelator, developed for gallium-68, is a good example of such a chelator. A variety of THP-peptide conjugates can be quantitatively radiolabelled with generator-produced gallium-68 at ambient temperature, in under two minutes at nanomole amounts of conjugate.^{26,37,40,41} This chemistry has underpinned development of the ⁶⁸Ga-labelled radiopharmaceutical [⁶⁸Ga]Ga-THP-PSMA (known as “Galliprost”) that targets PSMA and is prepared using a one-step kit at room temperature.²⁶ In a first-in-man trial, [⁶⁸Ga]Ga-THP-PSMA could identify primary and metastatic prostate tumors in PET scans, with lower uptake in off-target tissue than [⁶⁸Ga]Ga-HBED-PSMA.²⁷

Challenges in availability of radiometallic isotopes and imaging infrastructure

Although ⁶⁸Ga-radiopharmaceuticals are routinely available in many hospitals and PET clinics, a recent shortfall in supply of GMP-compliant ⁶⁸Ge/⁶⁸Ga-generators (relative to demand) has led to several centres being unable to supply [⁶⁸Ga]Ga-DOTA-octreotate (“Netspot”) for neuroendocrine patient PET scans.⁴² It is likely that ⁶⁸Ga-radiotracers for PSMA imaging will increase the demand for ⁶⁸Ga-generators. Whilst alternative methods of gallium-68 production (i.e. cyclotron production) and PET radionuclides (i.e. ¹⁸F-labelled PSMA-targeted radiopharmaceuticals⁴³) could potentially alleviate this shortage of radiotracers, new technetium-99m based radiopharmaceuticals have emerged that could also meet these clinical demands.

“Traditional” technetium-99m radiopharmaceuticals have been used in perfusion/functional imaging since the 1960s. Technetium-99m (half-life = 6.02 hours, γ 141 keV, 90%) is available from a ⁹⁹Mo/^{99m}Tc-generator, and technetium-99m remains the most widely available radionuclide for nuclear medicine. It is also worth noting that γ -scintigraphy and SPECT cameras are generally more readily accessible than PET facilities. For example, survey data collected in 2015 and 2016 across Europe (excluding the UK) indicated that there were 3408 γ -scintigraphy/SPECT cameras and 849 PET scanners.⁴⁴

Many ^{99m}Tc -labelled peptide- and protein-based radiotracers have been developed over the last few decades. Clinical development of ^{99m}Tc -labelled peptides and proteins in the 1990s and 2000s utilised HYNIC (6-hydrazinopyridine-3-carboxylic acid, Chart 1) derivatives.⁴⁵ In these radiopharmaceuticals, the HYNIC carboxylic acid group is used as an attachment point for targeting biomolecules and the 6-hydrazinopyridine group coordinates to $[\text{}^{99m}\text{Tc}]\text{Tc}^{\text{V}}$ in the presence of co-ligands such as EDDA (ethylenediamine-N,N'-diacetic acid), tricine or TPPTS (3,3',3''-phosphanetriyltris(benzenesulfonic acid)). The coordination environment of these technetium-99m conjugates remains undefined: HYNIC can potentially bind to $[\text{}^{99m}\text{Tc}]\text{Tc}^{\text{V}}$ in either a monodentate fashion through the hydrazine nitrogen only, or in a bidentate fashion where the pyridine nitrogen is also coordinated.^{46,47} It is possible that mixtures of such species form during ^{99m}Tc -radiolabelling. Despite this, several $[\text{}^{99m}\text{Tc}]\text{Tc}$ -HYNIC-peptides have been evaluated in the clinic. For example, $[\text{}^{99m}\text{Tc}]\text{Tc}$ -EDDA/HYNIC-octreotide ("Tektrotyd", Chart 3) is an alternative SSTR2-targeted agent for imaging neuroendocrine cancer patients.⁴⁸ It is conveniently prepared from an aqueous solution of generator-produced $[\text{}^{99m}\text{Tc}]\text{TcO}_4^-$ and a commercial two vial kit, containing HYNIC-octreotide conjugate, EDDA, tin chloride reducing agent, buffers and stabilisers. More recently, $[\text{}^{99m}\text{Tc}]\text{Tc}$ -3PRGD2 has shown promising diagnostic utility for lung, esophageal and breast cancer and rheumatoid arthritis.^{49,50} In $[\text{}^{99m}\text{Tc}]\text{Tc}$ -3PRGD2, a HYNIC conjugate, tricine and the TPPTS water-soluble phosphine all coordinate to the Tc metal center, and the HYNIC conjugate contains two copies of a cyclic RGD peptide that targets $\alpha_v\beta_3$ -integrin receptors expressed in neovasculature, inflammation events and many cancers. $[\text{}^{99m}\text{Tc}]\text{Tc}$ -3PRGD2 can be obtained in over 95% radiochemical yield, by adding an aqueous solution of $[\text{}^{99m}\text{Tc}]\text{TcO}_4^-$ to a single kit vial containing a lyophilised mixture of the HYNIC conjugate and all other reagents, followed by heating.⁵¹

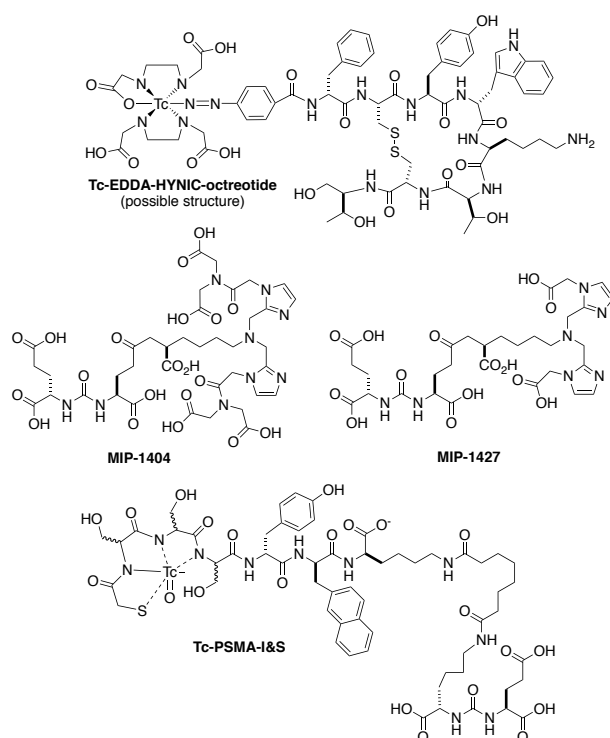


Chart 3. Chelator-peptide bioconjugates for ^{99m}Tc -radiolabelling

Although several ^{99m}Tc -labelled peptides/proteins have undergone clinical evaluation and even been commercialised, none have entered routine or widespread clinical use. However, recent innovations in ^{99m}Tc -labelled PSMA-targeted agents could potentially reverse this trend. Two classes of such radiopharmaceuticals have been developed. The first class consists of a PSMA-targeted peptide conjugate appended to a tridentate N₃ chelator consisting of a tertiary amine and two imidazole groups (MIP-1404 and MIP-1427), which can be radiolabelled in high radiochemical yield when reacted with the organometallic $\text{fac}-[\text{}^{99m}\text{Tc}][\text{Tc}(\text{CO})_3(\text{OH}_2)_3]^+$ complex, containing three labile water molecules. In these

procedures, two “kits” are required - the first to generate the $\text{fac-}^{99\text{m}}\text{Tc}[\text{Tc}(\text{CO})_3(\text{OH}_2)_3]^+$ complex from generator-produced $^{99\text{m}}\text{TcTcO}_4^-$, and the second to radiolabel the MIP conjugate.⁵² The resulting radiotracers, $^{99\text{m}}\text{Tc}[\text{Tc}(\text{CO})_3(\text{MIP-1404})]^+$ and $^{99\text{m}}\text{Tc}[\text{Tc}(\text{CO})_3(\text{MIP-1427})]^+$, have been used for both staging prostate cancer and assessing patients’ response to prostate cancer treatment,^{53–56} and $^{99\text{m}}\text{Tc}[\text{Tc}(\text{CO})_3(\text{MIP-1404})]^+$ has recently completed phase 3 clinical trials. The second radiotracer, $^{99\text{m}}\text{TcTcO}(\text{MAS}_3\text{-PSMA})$ (known as $^{99\text{m}}\text{TcTc-PSMA-I\&S}$), consists of a $^{99\text{m}}\text{Tc}$ -labelled peptide sequence, mercaptoacetyl-D-Ser-D-Ser-D-Ser, appended to the PSMA pharmacophore.⁵⁷ Three D-serine residues are used to increase the radiotracer’s metabolic stability. Unlike the $^{99\text{m}}\text{TcTc-MIP}$ -based radiotracers, this radiopharmaceutical can be formulated from a single kit (containing a tin chloride reducing agent, a weak chelator tartrate to stabilise reduced intermediate $^{99\text{m}}\text{Tc}^{\text{V}}$ species, buffer components and the chelator conjugate itself) in near-quantitative radiochemical yield. This radiotracer has entered the clinic: as well as demonstrating utility for SPECT imaging of prostate cancer, it has also been used in radioguided surgery.⁵⁸ One day prior to surgery, prostate cancer patients were administered $^{99\text{m}}\text{TcTc-PSMA-I\&S}$. During surgery, a gamma probe was used to facilitate localising metastases. These early studies demonstrate the successful “repurposing” of imaging agents as tracer agents for sensitive detection of disease in intraoperative surgery.

Challenges in antibody-based immunotherapies: understanding heterogeneity of disease

Monoclonal IgG antibody therapies have been transformative in the treatment of many cancers and autoimmune diseases. However, many patients treated with antibodies exhibit poor or heterogeneous responses. In some instances, this is likely due to heterogeneous expression of an antibody’s target receptor or heterogeneous antibody uptake at disease sites,^{59,60} which cannot be predicted or mapped from typical immunohistochemical analysis of single biopsies. The ability to quantitatively image antibody biodistribution at the whole-body level with PET imaging can help predict individual patient response, as well as aid in understanding treatment outcomes during clinical development. β^+ -emitting zirconium-89 (β^+ 23%, $E_{\text{max}} = 897 \text{ keV}$) has a half-life of 78 h, and like indium-111, this prolonged half-life is well suited for PET imaging of IgG antibodies. The increased availability of zirconium-89 (from cyclotrons) over the past decade has led to clinical development of highly sensitive zirconium-89 IgG PET imaging.

Clinical imaging with ^{89}Zr -labelled antibodies has used the siderophore, desferrioxamine-B (DFO, Chart 1), a natural product, produced and released by bacteria to sequester Fe^{3+} from the surrounding environment. DFO is a hexadentate O_6 chelator with three hydroxamate groups and is well suited for coordination of the very hard and oxophilic Zr^{4+} metal ion. In radiotracers and radiopharmaceuticals, DFO is derivatised at the primary amine with a reactive group that enables its covalent attachment to biomolecules. Whilst DFO derivatives have been developed that enable site-specific modification of IgG antibodies,⁶¹ the majority of recent studies have utilised DFO conjugates formed from reaction of a DFO-isothiocyanate derivative with primary amine groups of lysine amino acid side chains, to provide thiourea conjugates.⁶² These conjugates can be radiolabelled with aqueous solutions of zirconium-89 at room temperature and near-neutral pH, within 1 – 2 hours.⁶² In vivo, zirconium-89 remains bound to DFO and enables PET imaging of target tissue.

^{89}Zr -antibody imaging has been used to understand heterogeneous and variable responses to antibody-based cancer immunotherapies targeting immune checkpoint proteins, which allow tumors to evade the immune system. Atezolizumab targets the immune checkpoint PD-L1 receptor, and whilst atezolizumab is very effective in a sub-set of patients, many patients fail to respond to it. Recently, $^{89}\text{ZrZr-DFO-atezolizumab}$ has been administered to patients with bladder cancer, triple-negative breast cancer and non-small cell lung cancer, prior to the patients receiving a therapeutic dose of atezolizumab.⁵⁹ $^{89}\text{ZrZr-DFO-atezolizumab}$ PET scanning revealed significant inter-tumoral and intra-tumoral heterogeneity of $^{89}\text{ZrZr-DFO-atezolizumab}$ uptake (Figure 3). Importantly, $^{89}\text{ZrZr-DFO-atezolizumab}$ uptake corresponded to tumor response to atezolizumab therapy, and compared to immunohistochemistry of biopsies, PET imaging with $^{89}\text{ZrZr-DFO-atezolizumab}$ was significantly better at assessing whether patients would respond to antibody therapy.

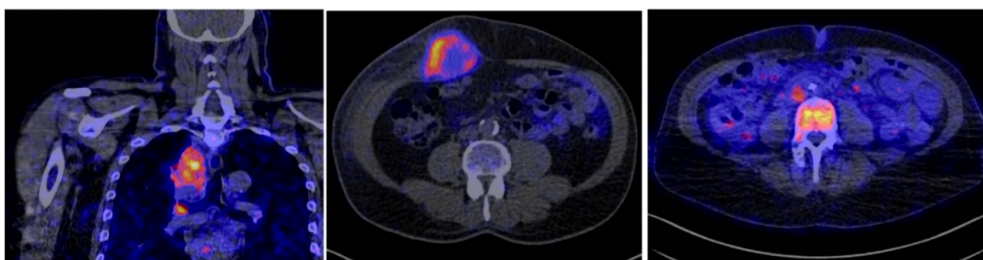


Figure 3: PET/CT images of lesions of three patients with heterogeneous intralesional [^{89}Zr]Zr-DFO-atezolizumab uptake on day 7 post-injection. Mediastinal lesion of a non-small cell lung cancer patient (left), an abdominal wall metastases of a bladder cancer patient (middle), and a bone metastasis of a triple-negative breast cancer patient (right). This research was originally published in *Nature Medicine*,⁵⁹ by Frederike Bensch et al., 2018;24:1852-1858 © SpringerNature.

Concluding remarks

Many new receptor-targeted biomolecular therapies are being developed, particularly in the field of oncology, and continuing research efforts will generate more. Ongoing molecular biology research will also lead to a better understanding of relationships between cell surface expression of particular receptors and disease progression. Finally, advances in radioisotope technology and nuclear physics will result in radiometallic isotopes with utility in PET/SPECT diagnostic imaging or radiotherapy. Clinical nuclear medicine will be able to integrate these advances to provide improved diagnostic imaging and therapeutic agents, provided that suitable and easy-to-use chelator derivatives exist. Such chelators and chelator-bioconjugates will be critical for the continued development, evaluation and translation of PET, SPECT and therapeutic radiopharmaceuticals based on new biomolecular and radioisotope technology.

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